

Towards Deciphering Molecular Factor Behind Higher level of 'Basmati' fragrance in *Pandanus amaryllifolius* Roxb., A wild monocot

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Abstract

The most important aroma component of scented rice, 2-acetyl-1-pyrroline (2AP) was earlier detected in 'pandan' leaves (*Pandanus amaryllifolius* Roxb.) from Thailand. Higher content of this volatile component was observed by gas chromatography later in *Pandanus* population from West Bengal, India and the wild plant was successfully cloned and maintained *in vitro*. The present study reports a molecular biological endeavor to decipher the high 2-AP induced aroma of the *Pandanus* clone. The molecular factor behind the aroma was conferred to allelic difference in Betaine Aldehyde Dehydrogenase (BADH2) gene in rice. Primers were synthesized from the sequences of badh2/Badh2 alleles of rice to obtain allele specific marker; and genomic DNA of *Pandanus* was tested in backdrop of certain scented and non scented rice varieties/landraces as check materials. A positive amplicon of 261 bp was observed in *Pandanus*, which was comparable to most of the scented rice varieties, while the amplicon size was 269 bp in case of the check non scented rice variety.

1. Introduction

Pandanus amaryllifolius Roxb., a member of Pandanaceae has been identified as a rich natural source of principle basmati aroma compound 2-acetyl-1-pyrroline (2AP). Quantitatively, the 2AP content is highest in *P. amaryllifolius* among the plant kingdom. Leaves of *P. amaryllifolius* are scented and exploited commercially by flavor industry. In India, *P. amaryllifolius* leaves are traditionally used for cooking common non aromatic rice to impart a resemblance of the basmati aroma to the cooked rice.

The molecular factor responsible for aroma in rice is conferred to the allelic difference in the BADH2 gene. Comparative studies on aromatic and non aromatic rice varieties reported till date infers that the wild type BADH2 gene does not lead to the accumulation of the aroma compound 2-acetyl-1-pyrroline while the altered/inactivated alleles of the BADH2 gene leads aroma (Bradbury et al. 2005). It was further understood that the wild form of this gene in its functional form do not result in aroma (non scented rice) while either a 7bp/8 bp deletion or a 3 bp insertion in different exons make this gene non-functional (Shi et al. 2008), which culminate in the expression of aroma (scented rice varieties). The present study reports a molecular biological endeavor to decipher the high 2-AP induced aroma of *Pandanus* in the backdrop of the information from previous studies in rice.

2. Experimental and Observations

2.1 Plant materials and Methods

In vitro maintained clones of high 2AP containing *Pandanus* from West Bengal, India were used as the experimental material (Gangopadhyay et al. 2004). Genomic DNA was isolated using Qiaquick DNeasy plant minikit (Qiagen) following the manufacturer's protocol. The DNA was subsequently quantified using Nanodrop Spectrophotometer (Thermo Scientific) and resolved in 1.0% (w/v) agarose gel to ascertain the quality of DNA. Additionally, DNA was also isolated from non aromatic (IR 64) and aromatic rice varieties (Pusa Basmati). Primers targeting specific aroma linked markers were synthesized following Shi et al. 2008:



Figure1. In vitro maintained Pandanus

Table1. Functional markers for BADH2 alleles (Shi et al. 2008)

<i>badh2</i> alleles	Sequence divergences	Functional markers	Primer sequences (5'-3')	PCR products
<i>badh2-E2</i>	A 7-bp deletion in exon 2	FM <i>badh2-E2A</i>	F: CCTCTGCTTCTGCCTCTGAT R: GATTGCGCGGAGGTACTTG	200/207 bp (<i>badh2-E2/Badh2</i>)
		FM <i>badh2-E2B</i>	F: CTCTGCCTCTGATTAGCCT R: GCCGTGAGCCATATACACTT	643/650 bp (<i>badh2-E2/Badh2</i>)
<i>badh2-E7</i>	An 8-bp deletion in exon 7	FM <i>badh2-E7</i>	F: GGTTCATTTACTGGGAGTT R: CAGTGAAACAGGCTGTCAAG	260/268 bp (<i>badh2-E7/Badh2</i>)

2.2 . Results:

The markers reported for various alleles of the BADH2 gene in rice were tested in *Pandanus*. Only one primer combination, which discriminates an 8bp deletion in exon 7 from the wild type BADH2 allele in rice showed promising results in *Pandanus*. An amplicon of 269 bp was observed in non scented rice (IR 64) while both scented rice (Pusa Basmati) and *Pandanus* revealed an amplicon of 261 bp.

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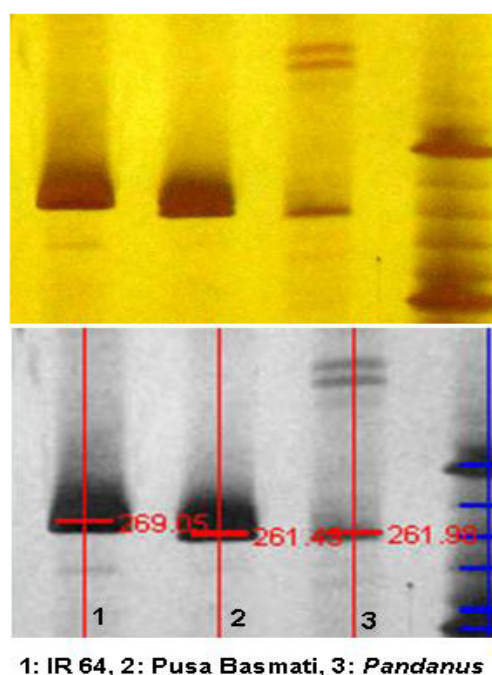


Figure1. Gel photograph and corresponding software aided molecular size analysis

3. Conclusions

A positive amplicon of 261 bp was observed in *Pandanus*, which was comparable to the scented rice variety; while the amplicon size was 269 bp in case of the check non-scented rice variety. This plausibly indicated an 8 bp deletion (as reported in exon 7 of rice). The amplicon is now awaiting sequence confirmation and software aided homology searching.

Additionally, the full length BADH2 gene of *Pandanus*, causing high amount of 2AP is also being investigated with a functional genomic approach taking cue from the partial cds of this gene in a recently deposited NCBI submission (GenBank: JQ067605.1).

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